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Molecular Dynamics Simulations of the Metaloenzyme Thiocyanate Hydrolase with Non-Corrinoid Co(III) in Active Site

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Thiocyanate hydrolase (SCNase, pdb code 2DD5) is a novel metaloenzyme containing non-corrinoid Co^{3+} in the active site. Despite identical structure of the active sites, high sequence and structural similarity of SCNase and nitrile hydratases (NHases) both enzymes catalyse different reactions. The SCNase enzyme catalyses the degradation of thiocyanate to carbonyl sulfide and ammonia but related NHases the hydration of nitriles to amides. The main goal of the present work was to explain these different properties on the molecular level. Extensive molecular dynamics simulations (up to 10 ns) were performed using CHARMM27 forcefield with specially designed parametrization of the active site. Particular attention was devoted to water dynamics in the catalytic region, dynamical properties of the entry channel and preferential docking sites for a substrate and products of SCNase. The theoretical modelling provides useful data for understanding this enzyme having properties desired in biotechnology.

1 Introduction

Thiocyanate hydrolase (SCNase) of *Thiobacillus thioparus* THI115 is a microbial metaloenzyme with non-corrinoid Co^{3+} metal ion in the non-standard active site^{1,2}. SCNase catalyses degradation of the thiocyanate to carbonyl sulfide and ammonia ($\text{SCN}^- + 2\text{H}_2\text{O} \rightarrow \text{COS} + \text{NH}_3 + \text{OH}^-$)¹.

SCNase is composed of three subunits α (15 kDa), β (18 kDa) and γ (28 kDa). In γ subunit it have non-standard active site composed of four residues: γCys128 , γCys131 , γSer132 , γCys133 . The γCys131 and γCys133 residues were found to be post-translationally oxidized to cysteine-sulfinic acid CysSO_2H (CSD) and cysteine-sulfenic acid CysSOH (CSO). The sequence analysis showed high similarity with quite extensively studied nitrile hydratases, especially between γ chain from SCNase and α chain from NHase. Crystallographic data show that the fold is similar to NHase and structures of the active sites are almost identical in these two enzymes¹. Main differences in the structure of active sites is that in NHase Co^{3+} ion is six-coordinated and in SCNase it is five-coordinated. The first five ligands are identical in both cases but in NHases the sixth coordination place it is occupied by a water molecule or a hydroxide ion. In SCNase the sixth coordination place is empty¹.

Although the structure of the SCNase is known, the structure does not contain either substrate nor products of reaction. So far only in one paper³ the interactions of the substrate and products with a mimetic center model were described. Knowledge about these interactions and dynamical properties of the SCNase can give answer about catalytical mechanism not only in thiocyanate hydrolase but also in NHase.

In this paper, for the first time, we describe docking studies of the substrate and products inside SCNase, Newtonian molecular dynamics of the enzyme and a variant of the MD

method - Locally Enhanced Samplig (LES) simulations with one of the products (NH_3) inside the protein matrix.

2 Methods

Docking was performed using the AutoDock 3.05 code⁴. Hydrogens were added into protein (chains G H I from the pdb file) using NAMD 2.6 psfgen tool⁵ and minimized in NAMD 2.6. Ligands were optimised using Gaussian03 code⁶ with the HF method and 6-31G* basis set. From these calculations also charges had been taken. Using AutoDock-Tools nonpolar hydrogens were merged and Gasteiger charges added. 256 runs of genetic algorithm (GALS) have been made for every ligand. The search grid covered the whole protein.

All molecular dynamics simulations for the enzyme were performed using NAMD 2.6 code. For the protein alone 11.5 ns long Langevin simulations in 300K with PBC have been obtained (preceded by 100ps equilibration of water box; $76 \times 66 \times 84 \text{ \AA}^3$, and 50 ps of heating up to 300K). LES⁷ simulations for the complex SCNase-ammonia were 5 ns long, with 10 copies of the ligand.

All analysis was performed using the VMD 1.8.6 code⁸ and home made scripts.

3 Results and Discussion

Docking of the product (SCN^-) and substrates (COS and NH_3) show that SCN^- and COS can directly bind to the metal ion (distance Co-N 2.7 Å and Co-O 2.83 Å respectively). In these two ligands many docks exhibit also coordination to βArg90 and βArg91 located above the modified γCys131 and γCys133 (3 Å). In the case of SCN^- only nitrogen atom interacts with these arginines, but COS is stabilized by sulphur and oxygen atoms. These docks are similar to nitrile docks in NHase described in our previous paper⁹. Ammonia does not exhibit direct coordination to the metal ion. The distance in the closest docks between Co^{3+} and nitrogen is 4.65 Å. The majority of docks "near" active site are very close to γCys131 , γCys133 and βArg91 . There also occur docks near γArg136 . We also observe docks in the channel leading to the active site, between βArg111 and βTrp112 . Docks closest to the active site are shown in Figure 1.

11.5 ns simulations of the enzyme without any ligand showed that the protein is stable. Maximum value of the RMSD was 1.9 Å. Fluctuations correlate well with B factors. In this type of the MD trajectory we focused on dynamical properties of water in the neighbourhood of the active site. Analysis showed that on the distance of 5 Å from Co^{3+} ion at least 40 waters molecules can be found. The data were collected from over 260000 counts. On distance 3 Å only 10 water molecules have been found and about 4900 counts. The shortening of the distance to 2.5 Å gives 7 water molecules and only 288 counts. This indicates that the active site is accessible for water molecules, but highly hydrophilic cavity causes that water molecules occupy space in some distance from the metal ion. Such behaviour may suggest that in the catalytical mechanism SCN^- coordinates to the metal ion and in this way it is activated for a water molecule attack. This is in accordance with docking results for the SCN^- .

The purpose of the LES simulations was to find a channel leading to the cavity. Ten non-interacting copies of the ammonia were located nearby metal ion. Two of them leaved

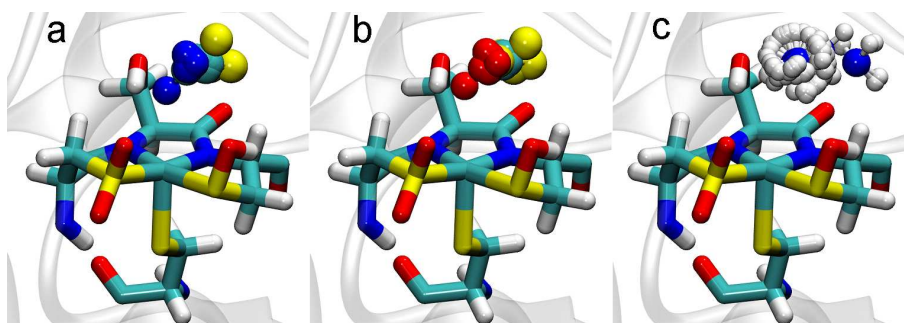


Figure 1. Docking places in the closest neighbourhood of the active site; SCN^- (a), COS (b) and ammonia (c).

the enzyme's interior. Paths of these ammonia molecules are shown in Figure 2a. The first molecule (Figure 2a black and 2b) passed to solvent after 0.7 ns. This trajectory seems to be not plausible because in the hetero-dodecameric structure this exit is closed by the other hetero-trimer and looks like "ballistic" one in the initial part of the simulation (rare artefact of the LES method). More probable is the trajectory shown in light grey (Figure 2a and 2c). In this case molecule leaved protein matrix after 1.9 ns. Contacts with residues in simulations are shown in Figure 2c. These two paths are quite different than those in NHase enzyme describes in our previous paper¹⁰.

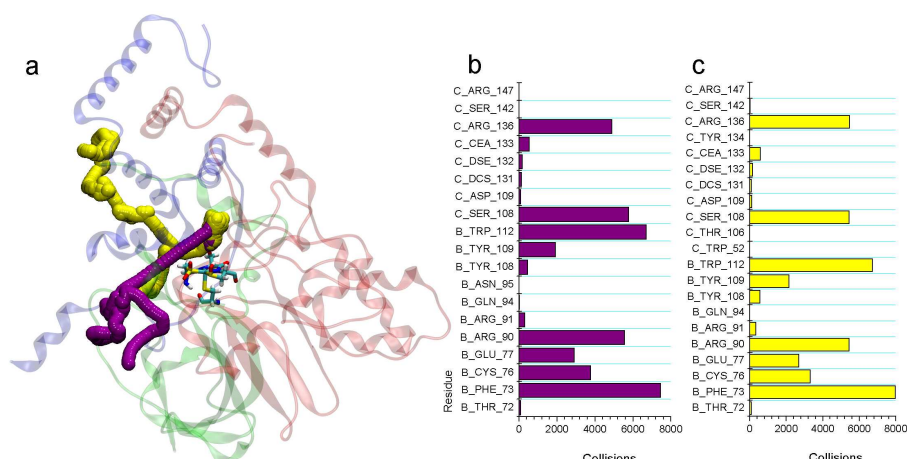


Figure 2. Trajectories of the two ammonia molecules (a), collisions counts of these molecules less plausible (b) and more plausible (c) in LES 10 simulations.

4 Conclusions

Docking studies on product and substrate show that the hypothetical catalytic mechanism relay on the binding of nitrogen atom from the SCN^- to Co^{3+} ion and such activated substrate is attacked by a water molecule. MD simulations show that a lot of water molecules are present in range of 5 Å from the metal centre. This is another hint for such type of the substrate activation. Through the LES simulation of the enzyme ammonia complex we obtained a realistic pathway of the product and indicated residues building the channel to the active site.

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